Residual Effect Of Certain Agrochemicals On The Total Soluble Protein And Specific Activity Of GOT And GPT Transaminases In The Earthworm, *Aporrectoda Caliginosa*

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ABSTRACT

The residual effect of the recommended and the 2-fold recommended rates of abamectin, flauzifop-p-butyl, lufenuron, mefenoxam plus and pymetrozine, in addition to the recommended rate of the mineral fertilizer (Monoammonium phosphate (MAP)) and the bio-fertilizer (Microbine) was determined on the adult and immature stages of the earthworm, *Aporrectoda caliginosa*. Most of the agrochemicals with 2-fold recommended rate caused harmful effects by decreasing the total soluble protein content of earthworm, while the recommended rate of treatments had little effect. In the adult stage, the 2-fold rates of fluazifop-P-butyl, lufenuron and mephenoxam plus caused the highest% reduction (50.77, 48.29, and 47.12), respectively. In the immature stage, pymetrozine, abamectin and mefenoxam plus caused 58.43, 53.30 and 53.07 % reduction, respectively. On the other hand, the results exhibited that the mineral fertilizer (M.A.P.) caused the highest reduction effect on protein content, in both adult and immature stages, of the earthworm among all used agrochemicals at their recommended rate revealing 44.20 and 40.48 reduction %. Meanwhile the recommended rate of the agrochemicals, with a little exception (MAP), caused transitional effects on GOT and GPT specific activity in both stages of earthworm. At the 2-fold recommended rate, it is clear that all the agrochemicals elevated the GOT and the GPT transaminases specific activity of adult and immature stages. In the adult stage, the highest increase of GOT specific activity was obtained by fluazifop-P-butyl, mefenoxam plus and abamectin (354.42, 223.15 and 213.84%), while, lufenuron and abamectin caused the highest stimulation (222.68 and 201.80%) of GPT at the 2-fold recommended rate. In the immature stage, the highest increase % of specific activity of GOT, was achieved by treatments of lufenuron, pymetrozine, abamectin and mefenoxam plus, at the 2-fold R. rate, showing the highest values as follows: 375.12, 303.69, 263.92 and 227.85%, respectively. The highest increase % of GPT specific activity with 2-fold R. rate recorded by treatments of pymetrozine, lufenuron, abamectin and mefenoxam plus (296.08, 230.99, 215.43 and 197.83%), respectively. Comparison between the two stages of the earthworm with respect to the effect of agrochemicals on the total protein content or GOT and GPT transaminases, the results indicated that, immature stage was more sensitive to these agrochemicals than adult stage. The treatment showed a degradable property of the tested pesticides where their effects are not persistent in the treated soil no more than 3 weeks.

INTRODUCTION

Earthworms have the major role in the breakdown of organic matter, releasing and recycling of nutrients. The physical effect of earthworms on soils was resulted from excavation of burrows and production of casts

(Sundaravadivel, 1998). Moreover, earthworms play a great role in the fertility and productivity of cultivated soils as cleared by many authors (Edwards, 1965, Van Rhee, 1965 and Anne-Grace, 1996). In addition, some scientists clarified that earthworms played a role in the humification of the organic matter and productivity of soil, so the earthworms are considered one of the members in the series leading to the formation of humus.

The widely used of pesticides has created a biotic factor which affecting almost every ecosystem and are extremely toxic to earthworms. Many studies have shown that they caused marked decrease in the earthworm populations when applied to varieties of terrestrial ecosystems. These were occurred due to the condensed agricultural production which required heavy use of pesticides to alleviate countless pests (Drewers and Zoran, 1987 and Maurya and Chattoraj, 1994).

However, the transaminases GOT and GPT have a role towards the utilization of proteins and carbohydrates. In addition, these enzymes are involved in the breakdown of amino acids into α-keto acids, which are responsible for metabolism through the Krebs-cycle as well as electron transport chain. Liver tissue in mammals is rich in GPT. Therefore, GPT content determination is more important than GOT. It is reported that certain pesticides were effective against the activity of enzymes in earthworm indicating detrimental effect. So, earthworm could be used as "biomarker" to evaluate the soil pollution [Edward and Lotfy. 1977, Chabert Fayolle (1995), Ismail *et al.* ($1997_{a, b}$) and Mosleh *et al.*, ($2002_{a, b}$)]. The safety of pesticides is of a great importance to avoid unwanted side influence either on earthworm or on structure, composition and fertility of soil (Stenersen and Quien, 1980).

Little information is available about the use of earthworms as biochemical indicator of environmental contamination (Lofs-Holmin, 1983; Pizl, 1988 and Edwards and Bohlen, 1995). The present study was initiated to estimate the influence of certain recent agrochemicals (certain pesticides and fertilizers) on the total soluble protein and the activity of GOT and GPT enzymes of the earthworm "*Aporrectoda caliginosa*" (adult and immature stages). Furthermore, to reveal the best range of these agrochemicals could be compatible with IPM program.

MATERIALS AND METHODS

Agrochemicals used:

A.) Pesticides used:

1) Abamectin (Vertimec 1.8% EC) ^{R} (bio-insecticide and acaricide).
2) Lufenuron (Match 5% EC) ^{R} (Chitin synthesis inhibitor).
3) Pymetrozine (Chess 5% EC) ^{R} (insecticide).
4) Fluazifop -P- butyl (Fusilade Super 12.5% EC) ^{R} (Herbicide).
5) Mefenoxam plus (Ridomil Gold Plus 42.5 % WP) ^{R} (systemic fungicide).

B) Fertilizers used:

1) Microbine ^{R} [Commercial multi nutrient (biofertilizer)]: mixture of P-dissolving and N_2 – fixing bacteria (Azospirillum, Azotobacter, Klebsiella and Bacillus ...etc).
2) Mono Ammonium phosphate (mineral fertilizer): Chemical name: Ammonium dihydrogen phosphate (NPK 12–60– 0).

The commercial formulations of the tested pesticides and fertilizers were used in this study are according to Egyptian Agriculture Ministry recommendations. The pesticides used in their aqueous dilutions were sprayed on the tomato plant surface using a pot sprayer. All rates were calculated according to soil surface area of the experimental pots. The used recommended rates of the pesticides were 40 ml/100L, 1000 ml/feddan area, 160 ml/feddan area, 800 gm./feddan and 240 gm/feddan area for Vertimec, Fusilade super, Match, Ridomil Gold plus and Chess, respectively.

The pesticides as previously shown with two rates of application (the recommended and 2-fold recommended rates) were applied on the vegetative tomato plants. The moisture content of soil was adjusted to 60% of its water holding capacity (WHC) and was maintained during the course of study.

As for the bio fertilizer (Microbine); the recommended rate (500 gm/feddan) was added beside the plant roots, covered with a little of soil, while the recommended rate of the inorganic fertilizer (Mono- ammonium phosphate 200 kg /feddan), was added onto the surface of the experiment pots. The experimental pots were then irrigated just after fertilization.

Preparation of soil samples:

A sandy loam soil was collected from free pesticides area attached to Agricultural Research Station, Sabaheia region, Faculty of Agric. Farm, Alex. Univ., Alexandria Governorate. Soil samples were air–dried, crushed and sieved through a 2-mm screen. Soil samples were placed in perforated black plastic pots (2 kg capacity and 20.0 cm depth each), mixed thoroughly with dried organic manure (0.5 kg fertilizer/100kg soil), sown

with broad bean seeds to activate the soil and sown another time with tomato seeds (Castlerock variety-5August 2003) which were mixed with (Phosphorine) biofertilizer.

Rearing technique of the earthworm:

Earthworm, *Aporrectodae caliginosa* CARISTOTLE (B.C), adult and immature stages were collected by hand sorting methods from agricultural fields located at El-Tarh and Tabia regions, Alex. Governorate. These earthworms were identified according to Zoology Department, Faculty of Science, Alex. Univ. (Moursi and Dekinesh, 1984 a, b). Earthworms were reared according to the methods of Moursi and Dekinesh (1975 a, b).

The earthworm adults were transferred onto soil surface, then the pots were covered by mousseline cloth which were closely tighten by rubber ring to prevent worms from escaping and allow ventilation, then placed them into metallic cage (2 m X 1.0 m X 0.3 m). The pots were maintained at laboratory conditions for successive generations where, several eggs are laid into the cocoon, but after an interval of about 12 weeks usually only a single young worm emerges form the cocoon.

Performance of the earthworm treatments:

Thirty days after sowing, tomato plants were treated with the investigated agrochemicals according to the above mentioned application programs. The water content of soil-pots were kept to be at 80-90 % saturation value. Therefore, an adequate amount of water was added to compensate the water loss during the study course.

The experimental pots were divided into four groups. The 1st group was treated with the pesticides and fertilizers, the 2nd, 3rd and 4th groups were treated 1, 2 and 3 weeks after the treatment of the first group, respectively. Afterwards, 10 adults + 10 immature stages of earthworms (*Aporrectoda caliginosa*) were placed on the top of each pot, including also the "untreated" check.

The experimental pots were covered by fine gauze, closely tighten by rubber ring to prevent worms from escaping and allow ventilation and then incubated at 25 ± 1°C, 80-90 % R.H. Three replicates were used for each treatment. After one week of incubation, the percent mortality values were recorded and then corrected by Abbott's formula (1925). The body weight values of alive worms were recorded and the total soluble protein of earthworms tissue were assessed. Also, the activity of both GOT Glutamic Oxaloacetic transaminase (AST) and GPT Glutamic Pyruvic transaminase (ALT) enzymes was determined.

Biochemical assay:

One gram of earthworm was homogenized in 10 ml of 0.2 mol/L phosphate buffer (pH 7.4) using Polytrone Homogenizer. The crude homogenate was centrifuged under cooling (-4 °c) for 20 min. at 6000 xg (5500 rpm). The obtained supernatant was subjected to total protein determination (at 540 nm) according to Gornall *et al.* (1949). The color absorbance was determined by the Spectronic 21 D spectrophotometer.

The activity of GOT and GPT enzymes was determined spectrophotometrically (at 530:550 nm) in the supernatant of treated earthworms, according to Retiman and Frankel (1957) using commercial product kits supplied from Diamond Co., Egypt. The activity of GOT and GPT enzymes is expressed as unit per liter of the earthworms homogenate (U/L) referring to kit- includes conversions.

RESULTS AND DISCUSSION

1- Effect of agrochemicals on total soluble protein of earthworm, adult stage of *A. caliginosa.*

Data shown in Table (1) indicate the effect of the pesticides at the recommended and the two fold recommended rates as well as the recommended rate of Monoammonium phosphate and Microbine on both soluble protein and reduction percent of soluble protein of the adult stage of ***A. caliginosa.***

At the recommended rate, the results showed that certain treatments gave a little decline in protein content while others induce intensive reduction. The highest significant decrease was induced by M.A.P. (4.54) at the 1st week, microbine, M.A.P. and abamectin (12.39, 12.78, 13.06 mg protein/ml homogenate) at the 2nd week. The decreasing effect was continued at the 3rd week by all treatments and disappeared at the 4th week except by M.A.P.

At the 2-fold R. rate, the agrochemicals intensively decreased the soluble protein content. After the 1st week of treatment, the highest decrease was achieved by abamectin (3.38 mg protein/ml homogenate supernatant). Pymetrozine was the most effective in reducing the protein content after the 3rd period of evaluation followed by lufenuron and mefenoxam plus when compared with the check, where the protein content values were 4.12, 4.16, 4.23 compared with 13.44 in check mg protein/ml earthworm homogenate, respectively.

The general means of the protein level effect indicated that the agrochemicals clearly decreased the protein content of earthworm tissue. The treatments showed that fluazifop-P-butyl at 2-fold R. rate caused high

reduction on soluble protein content of earthworm tissue, followed by lufenuron and mefenoxam plus at the same rate of application, where the values were 6.75, 7.09 and 7.25 mg protein/ml homogenate, respectively, while the untreated check was 13.71. Lufenuron and pymetrozine at the recommended rate showed 11.11, 11.48 mg/ml homogenate showing no significantly differences than control. On the other hand, according to the use of the recommended rate, the significant reduction in the total soluble protein was achieved by both used fertilizers (Monoammonium phosphate, (7.65) and Microbine, (10.01) followed by abamectin (10.28), mefenoxam plus (10.37) and fluazifop-P-butyl (10.92 mg/ml homogenate), consecutively. These findings showed that certain treatments with recommended rates gave a little decline in protein content of the earthworm body weight in soil plots while others induce intensive reductions.

Table(1). Effect of certain agrochemicals on the total protein of adult stage of earthworm *A. caliginosa*.

Agro-chemicals	Rate	Protein level (mg/ml) at different weeks				
		1st week	2nd week	3rd week	4th week	Mean
lufenuron	R	7.98 de ±0.2	15.76 a ± 0.7	5.42 cd ± 0.2	15.26 a ±1.3	11.11 ab
	2 R	7.49 e ± 0.1	11.01 cd± 0.6	4.16 d ± 0.1	5.69 ef ±0.3	7.09 e
pymetrozine	R	14.41 a ±0.7	14.19abc ±0.6	9.01 b ± 0.9	8.31 cd ± 0.4	11.48 ab
	2 R	12.83 b ± 0.8	14.03 cd ± 1.1	4.12 d ± 0.2	4.40 f ± 0.2	8.84 cde
abamectin	R	9.08 cd ±0.1	13.06bcd ±0.8	8.34 b ±0.5	10.64 b ± 0.8	10.28 bcd
	2 R	3.38 h ± 0.0	13.34abc± 0.4	6.21 c ±0.2	7.80d ± 0.7	7.58 de
fluazifop-p-butyl	R	9.24 c ± 0.0	15.22 a ±0.4	9.12 b ±0.5	10.11 bc ± 0.5	10.92 b
	2 R	6.13 f ±0.0	9.78 d ± 0.9	6.18 c ± 0.6	4.89 f ±0.1	6.75 e
mefenoxam plus	R	9.46 c ±0.0	14.95 a ± 0.7	6.87 c ± 0.2	10.21 b ±0.2	10.37 bc
	2 R	5.64 fg ±0.0	11.29 cd ± 1.1	4.23 d ± 0.2	7.83d ± 0.7	7.25 e
M.A.P.	R	4.54 g ± 0.1	12.78bcd± 0.7	6.22 c ± 0.2	7.05 de ± 0.3	7.65 de
Microbine	R	12.52 b ± 0.19	12.39bcd±0.2	6.35 c ± 0.3	8.77 bcd ± .6	10.01 bcd
Check	--	14.75a ± 0.8	16.72a ± 0.4	13.44 a ±1.1	9.88 bc ± 1.1	13.71 a

* In a column, means followed by the same letter(s) are not significantly different at 0.05 probability level.
* R: Recommended rate * 2 R: 2-fold recommended rate * M.A.P.: Mono ammonium phosphate

Data in Fig. (1) illustrates that the percent reduction, throughout the whole study period, on the total soluble protein revealed that the agrochemicals had deleterious effect on total soluble protein especially at the 2-fold rates, where and fluazifop-P-butyl, lufenuron and mephenoxam

plus caused the highest% reduction (50.77 48.29, and 47.12), respectively. The results exhibited that the mineral fertilizer (M.A.P.) caused the highest depression on protein content of earthworm among all the used agrochemicals at their recommended rate, where the reduction % were 44.12, 26.99 and 25.02 % for M.A.P, Microbine and abamectin, respectively. It is obvious that the least deleterious agrochemical of the used ones is pymetrozine at both used rates.

The present results are similar to the data reported by Ahmed *et al.* (1991) who found that decreasing the growth rate and reduction of soluble protein of earthworm were correlated to the increase of diflurobenzuron concentrations. Ismail *et al.*(1997_a) showed that residues of atrazine caused a significant effect on total soluble protein content of earthworm *A.caliginosa.*, Ismail *et al.*(1997_b) indicated that the previous pesticide caused significant decline of soluble proteins content of *A. caliginosa.* Mosleh *et al.* ($2002_{a,b}$, 2003) found that both aldicarb and endosulfan significantly reduced the growth rate and the total protein content of earthworm, *L. terrestris.*

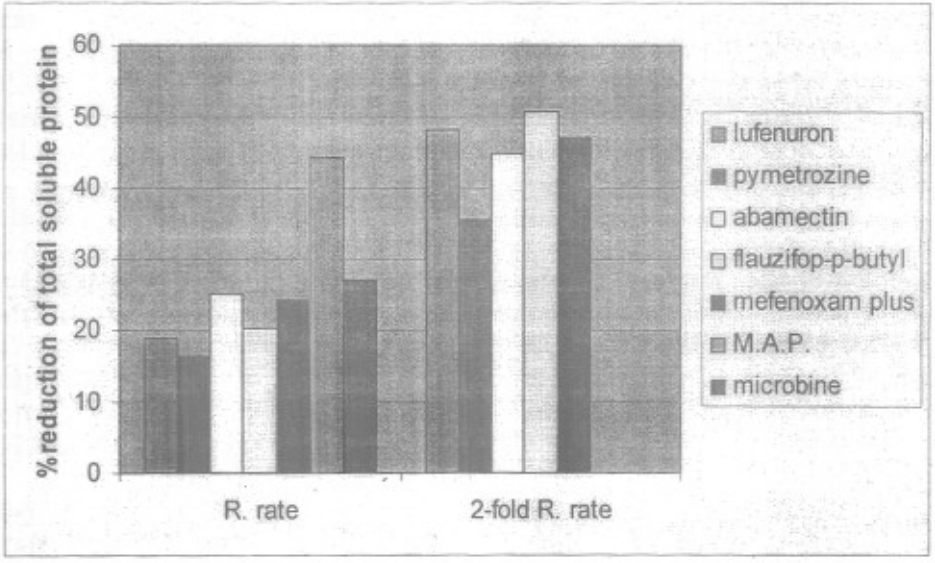

Fig. (1): Residual effect of certain agrochemicals on % reduction of total protein of earthworm, adult stage of *A. caliginosa*

2- The effect of agrochemicals on total soluble protein of earthworm, immature stage of *A.caliginosa:*

Data shown in Table (2) and Fig. (2) revealed that the agrochemicals decreased the soluble protein content of immature stage of earthworm tissue. As regards to the recommended rate, pymetrozine and M.A.P.induced significant decrease throughout the sequential four week-intervals. Whereas, the least content was achieved by pymetrozine (8.15) in the 1st week and by M.A.P. and pymetrozine (5.43 and 5.62 mg/ml) in the 3rd week. Moreover, at the 2-fold R. rate the least total protein content was related to pymetrozine (3.71 and 3.44 mg protein/ml homogenate) after the 3rd and the 4th week, respectively. While, abamectin recorded (4.78) more decline after the 3rd week of application. It is obvious that the harmful effect of both used rates of treatment increased gradually and reached the maximum effect at the 3rd week post-treatment, then began to decrease at the 4th week.

The calculated general means of the total soluble protein, throughout the four successive weeks of investigation post-treatment, indicated that the agrochemicals decreased the protein content of the earthworm tissue. Pymetrozine at the two fold R. rate caused the highest reduction in soluble protein content (5.35) followed by abamectin, mefenoxam plus and lufenuron at the same rate of application (6.01), (6.04) and (6.31), respectively compared with 12.87 mg protein/ml homogenate in untreated check. Regarding to the recommended rate, MAP induced the least soluble protein content (7.66 mg/ml) followed by pymetrozine (7.87 mg/ml).

As for the reduction % on the total soluble protein of immature earthworm, the 2-fold R. rate of treatments for pymetrozine, abamectin and mefenoxam plus caused 58.43, 53.30 and 53.07%, respectively. At the recommended rate, M.A.P. and pymetrozine achieved the highest reduction% comprising 40.48 and 38.85%, respectively. Noticeably, the least harmful agrochemicals to the soluble protein in the immature stage of the earthworm were Microbine, flauzifop-p-butyl and lufenuron among those used at the recommended rate, revealing 18.18, 20.59 and 23.23% of reduction, consequently (Fig. 2).

It could be concluded that most of the treatments with 2-fold recommended rate caused harmful effects to the total soluble protein of earthworm, while the recommended rate of treatments had little effect in comparison with check (untreated). The residual effect of lufenuron, abamectin, fluazifop-P-butyl and mefenoxam plus showed an initial effect of decreasing the soluble protein then became transient at the 4th week of the treatment showing a degradable property of these pesticides and they are not persistent pesticides in the used soil not more than

Table (2). Effect of certain agrochemicals on the total protein of the immature stage of earthworm *A. caliginosa*

Agrochemicals	Rate	Protein level (mg/ml) at different weeks				
		1st week	2nd week	3rd week	4th week	Mean
lufenuron	R	15.48 a ± 0.1	8.63 d ± 0.3	7.31 a ± 0.9	8.09 b ±0.4	9.88 bc
	2 R	7.57 f ± 0.3	6.80 e ±0.5	5.26bcd ± 0.6	5.62 e ±0.3	6.31 ef
pymetrozine	R	8.15 ef ± 0.3	9.42 cd ± 0.2	5.62 bcd ± .3	8.27 b ± 0.9	7.87 de
	2 R	6.34 g ± 0.5	7.91 de ± 1.1	3.71 d ± 0.1	3.44 f ±0.1	5.35 f
abamectin	R	9.20 cd ± 0.0	10.89 c ± 0.5	7.37 a ± 0.7	7.78b ± 0.8	8.81 bcd
	2 R	5.86 g ±0.0	7.97 de ± 0.4	4.78 cd ± 0.0	5.49 e ± 0.4	6.01 ef
fluazifop-p-butyl	R	8.64 de ± 0.1	13.52 b ± 0.8	8.10 a ± 0.6	10.60 a ± 0.7	10.22 bc
	2 R	8.25 ef ± 0.0	9.64 cd ± 0.9	7.55 a 0.4	5.79 de ± 0.1	7.81 de
mefenoxam plus	R	9.72 c ± 0.0	9.13 cd ± 0.4	8.23 a ±0.8	7.42 bcd ± .0	8.63 cd
	2 R	6.41 g ± 0.0	6.60 e ± 0.8	5.10 cd ± 0.7	6.03 cde ±0.0	6.04 ef
M.A.P.	R	9.69 c ± 0.1	9.71 cd ± 0.3	5.43 bc ± 0.6	5.82 de ± 0.5	7.66 de
Microbine	R	14.43 b ± 0.5	13.17 b ± 0.1	6.93 ab ± 0.2	7.59 bc ± 0.5	10.53 b
Check	–	14.38 b ± 0.2	16.88 a ± 0.9	8.47 a ±0.8	11.76 a ± 1.2	12.87 a

* In a column, means followed by the same letter(s) are not significantly different at 0.05 probability level.
* R:Recommendedrate * 2 R:2-fold recommended rate* M.A.P.:Mono ammonium phosphate

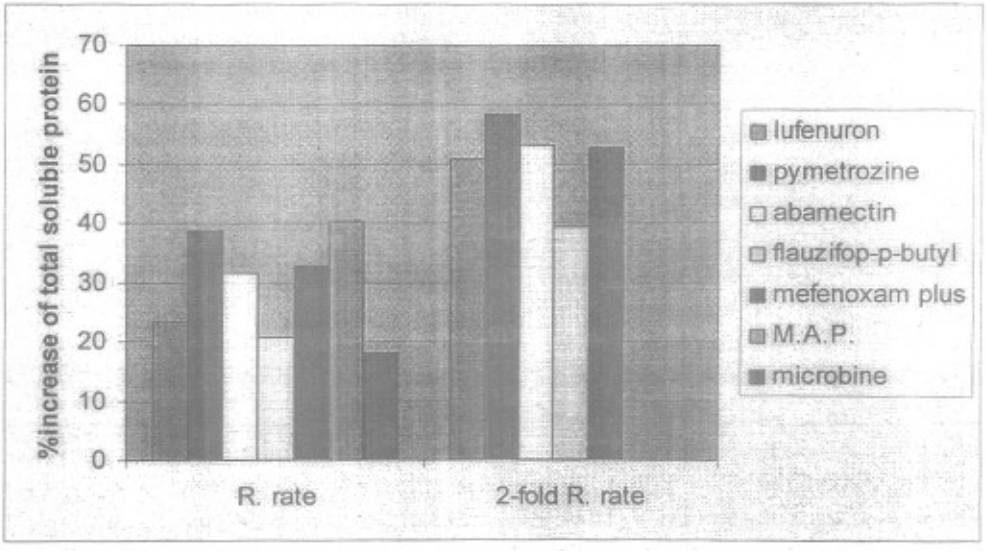

Fig. (2): Residual effect of certain agrochemicals on total soluble protein of earthworm, immature stage of *A. caliginosa*

Comparison between the two stages of the earthworm with respect to the effect of agrochemicals on the total protein content, the results indicated that, immature stage was more sensitive to these agrochemicals than adult stage. These finding results are in line with reported by Ahmed *et al.*(1991), Ismail *et al.*($1997_{a,b}$) and Mosleh at *et al*(2002a,b), (2003).

3) Effect of certain agrochemicals on the activity of the transaminase GOT (AST) & GPT (ALT) of earthworm, adult stage of *A. caliginosa* in soil.

Results in Table (3) indicated that GOT specific activity in *A.caliginosa* was high in agrochemical treated soil plots especially at the 2-fold recommended rate after the 3rd week of treatment. Where the highest values were occurred by fluazifop-P-butyl followed by M.A.P., pymetrozine, mefenoxam plus, lufenuron and abamectin 20.20, 20.09, 16.84, 16.54, 13.01 and 12.57 U/L/mg protein/min, respectively. However, the highest increase of specific activity of GOT, along the sequential four weeks, was occurred by abamectin (19.14) at 2-fold R. rate after the 1st week of treatment, and the highest increase (36.91 U/L/mg protein/min) obtained by fluazifop-P-butyl at the double rate after 4 weeks of application.

Means of GOT specific activity, among both rates of application, could significantly be arranged as follows: fluazifop-P-butyl (19.04) at 2-fold R. rate > mefenoxam plus (13.54) at 2-fold R. rate = abamectin (13.15) at the double rate = M.A.P. (12.43) ≥ pymetrozine (11.85) at 2-fold R. rate > lufenuron (11.59) at the double rate > fluazifop-P-butyl (8.48) at R. rate > abamectin (6.58) at R. rate = lufenuron (6.46) at normal rate = mefenoxam plus (5.86) at R. rate = pymetrozine (5.21) at R. rate = Microbine (5.16) ≥ check (4.19). It could be concluded that the used agrochemicals, at their R. rates, did not differ significantly than the untreated check; except the treatment of M.A.P. and fluazifop-P-butyl. On the other hand, all of the pesticide treatments, at their 2-fold R. rates differed significantly than untreated check.

The same trend was shown with the increase % of the mean of GOT specific activity values (Fig,, 3) during the whole period of the four successive weeks, where fluazifop-P-butyl, mefenoxam plus, abamectin and lufenuron at the 2-fold R. rate recorded the highest values as follows: 354.42, 223.15, 213.24 and 176.61%, respectively. However, the R. rate of the agrochemicals caused a little effect on GOT specific activity except M.A.P. and fluazifop-P-butyl (196.66 and 102.39%). It is worth to note that, the biofertilizer (Microbine) proved more safe than mineral fertilizer (M.A.P.).

GPT enzyme activity affected by the tested agrochemicals was illustrated in Table (4) & Fig. (3). The agrochemicals caused stimulation of GPT specific activity during the evaluation periods showing that abamectin at 2-fold R. rate caused the highest increase of GPT specific activity (10.66 U/L/mm protein/min) after 1 week of application. At the 2nd and the 3rd weeks, fluazifop-P-butyl and mefenoxam plus at 2-fold R. rate caused the highest increase of GPT specific activity (4.68 and 7.98 U/L./mg protein/min), respectively. Moreover, lufenuron (at 2-fold R. rate) caused the highest increase (8.86) after the 4th week.

At the end of experiment, it was found that the mean of the specific activity of GPT (U/L/mg protein/min) could be arranged as follows: lufenuron at 2-fold R. rate (5.83) ≥ abamectin at 2-fold R. rate (5.46) ≥ fluazifop-P-butyl at the double rate (5.40) ≥ mefenoxam plus at 2-fold of the normal rate (4.46) ≥ pymetrozine at the double rate (3.89) ≥ M.A.P. (3.45) ≥ Microbine (2.74) = abamectin at R. rate (2.63)= mefenoxam plus at the recommended rate (2.58) =fluazifop-P-butyl R. rate (2.46) = pymetrozine at the normal rate (2.36) = lufenuron at the recommended rate (2.33) = check (1.81).

Table (3). Residual effect of certain agrochemicals on GOT specific activity of earthworm, adult stage of *A. caliginosa*

Agrochemicals	Rate	Specific activity (U/L/mg protein/min) at different week- intervals					
		1st week	2nd week	3rd week	4th week	General mean	% increase of general mean
lufenuron	R	7.26 e ±0.5	4.29cd± .2	8.78de± .4	5.51ef ±0.4	6.46 e	54.18
	2 R	11.94b±0.8	5.78 b ±0.3	13.01c± .6	15.62cd±.9	11.59 bc	176.61
pymetrozine	R	3.56hi ±0.2	5.09bc±0.1	5.41fg ±0.4	6.77ef ±0.5	5.21 de	24.34
	2 R	4.81fg± 0.3	6.18 b ±0.4	16.84b±0.6	19.57b±0.4	11.85 bc	182.82
abamectin	R	5.14 f ± 0.1	5.48bc±0.4	9.04 d ±0.4	6.66ef ±0.4	6.58 de	57.04
	2 R	19.14a± .5	5.87 b ±0.3	12.57c± .2	15.0cd±1.3	13.15 b	213.84
fluazifop-p-butyl	R	5.81f ±0.01	5.03bc± .2	8.78de±0.8	14.28d±1.2	8.32 cd	102.39
	2 R	9.66 d± 0.1	10.10a±0.9	20.20a±1.4	36.19a±0.4	19.04 a	354.42
mefenoxam plus	R	4.71fg ±0.1	4.23cd±0.2	6.93ef ±0.0	7.56ef ±0.6	5.86 de	39.86
	2 R	11.15c±0.1	9.33 a ±0.5	16.54b±1.1	17.15c± .2	13.54 b	223.15
M.A.P.	R	12.94b±0.5	8.87 a ±0.5	20.09a±0.8	7.80 e ±0.4	12.43 b	196.66
Microbine	R	4.24gh±0.1	3.73 d ±0.2	6.80 f ± 0.3	5.87ef ±0.5	5.16 de	23.15
Check	–	3.07 i ± 0.3	3.50 d ±0.3	4.79 g ±0.3	5.40 f ± 0.6	4.19 e	–

* In a column, means followed by the same letter(s) are not significantly different at 0.05 probability level.

* R: Recommended rate * 2 R: 2-fold recommended rate * M.A.P.: Mono ammonium phosphate

Table (4). Residual effect of certain agrochemicals on GPT activity of earthworm, adult stage of *A. caliginosa*

Agro-chemicals	Rate	Specific activity (U/L/mg protein/min) at different week- intervals					
		1st week	2nd week	3rd week	4th week	General mean	% increase of general mean
lufenuron	R	1.81 g± 0.0	2.20 d± 0.1	2.79 e± 0.1	2.53 d± 0.2	2.33 b	29.05
	2 R	5.19 c± 0.1	3.42 c± 0.3	5.86 b± 0.2	8.86 a± 0.5	5.83 a	222.68
pymetrozine	R	1.23 i± 0.1	3.61 bc±0.2	1.62 f± 0.1	3.19 cd±0.2	2.41 b	33.47
	2 R	1.51 h± 0.1	4.29ab±0.5	3.47 de±0.4	6.27 b± 0.3	3.89 ab	114.94
abamectin	R	2.79 e± 0.1	3.59 bc±0.3	1.59 f± 0.1	2.54 d± 0.2	2.63 b	45.37
	2 R	10.66 a±0.1	3.81 bc±0.1	3.69 cd±0.1	3.66 c± 0.3	5.46 a	201.80
fluazifop-p-butyl	R	1.43 hi±0.0	2.26 d± 0.1	3.6cde±0.2	2.54 d±0.2	2.46 b	36.10
	2 R	5.73 b± 0.0	4.68 a± 0.4	5.67 b± 0.6	5.53 b± 0.2	5.40 a	198.89
Mefenoxam plus	R	0.97 k± 0.0	2.35 d± 0.0	4.74 c± 0.1	2.58 d± 0.2	2.58 b	42.46
	2 R	2.55 f± 0.0	4.13 bc±0.4	7.98 a± 0.6	3.18cd± 0.2	4.46 ab	146.78
M.A.P.	R	3.43 d± 0.1	2.54 d± 0.1	4.33 cd± .2	3.51 c± 0.2	3.45 ab	91.01
Microbine	R	1.26 j ± 0.0	2.56 d± 0.0	3.91 cd±0.4	3.23cd± 0.2	2.74 b	51.59
Check	–	0.94 k± 0.0	2.17 d± 0.0	1.59 f± 0.1	2.53 d± 0.3	1.81 b	–

* In a column, means followed by the same letter(s) are not significantly different at 0.05 probability level.
* R:Recommended rate *2R: 2-fold recommended rate *M.A.P.: Mono ammonium phosphate

The reduction % of GPT specific activity increased due to the treatment of the agrochemicals, whereas, abamectin, lufenuron, abamectin and fluazifop-P-butyl at two fold R.rate recorded the highest values as follows: 221.68, 201.80 and 198.89 %, respectively. However, the least deleterious pesticides, in this respect, is pymetrozine at its both tested rates (Fig. 3).

Moreover, according to mean activity of enzymes and the reduction percents during 4 weeks, the elevation of the specific activities of both GOT & GPT enzymes was a little significance by the application of recommended rate in comparison with the check, except M.A.P. and fluazifop-P-butyl for GOT and/or M.A.P. for GPT. On the other hand, all tested agrochemicals, at the 2-fold R. rate, significantly increased the activity of the two enzymes indicating that GPT was higher elevated than GOT enzyme.

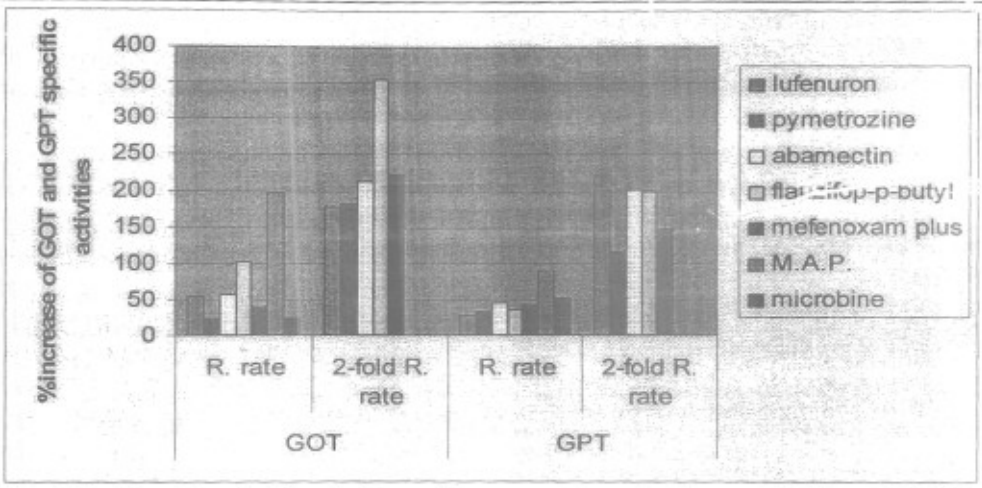

Fig. (3): Residual effect of certain agrochemicals on GOT and GPT specific activity of earthworm, adult stage of *A. caliginosa*

Reviewing the obtained results, it could be proved a negative relation between the activity of the studied enzymes and the soluble protein content. The present observation are similar to the data reported by Ahmed *et al.*(1991), Ismail ($1997_{a,b}$) and Beltagy (2000) who showed a parallel effect between the reduction of growth rate, the decrease of total soluble protein, and the elevation of transaminases activities.

4) Residual effect of agrochemicals on the activity of the transaminases enzymes, GOT (AST) and GPT (ALT) of earthworm, immature stage of *A. caliginosa* in soil.

Results in Table (5) and Fig. (4) showed that GOT specific activity in immature worms treated with agrochemicals especially at the double rate was higher than that of the check (untreated) where the highest values were occurred by lufenuron after 1, 2 and 3 weeks (16.54), (18.52) and (19.41) U/L/mg protein/min, respectively. However, after the 4th week of treatment, pymetrozine at the same rate of application recorded the highest value among all agrochemicals or the inspected period, (33.52 U/L/mg protein/min). Also, after 2nd week of treatment, abamectin at the 2-fold R. rate recorded high elevation on GOT specific activity, (19.76 U/L/mg protein/min).

It is clearly evident to note that, the agrochemicals involved Microbine, abamectin, fluazifop-P-butyl, lufenuron, mefenoxam plus, and pymetrozine had a slight effects on the general means of GOT specific activity at their recommended rate in comparison with check (5.59, 5.79, 5.82, 5.95, 6.38, and 7.02), respectively. On the other hand, all the tested pesticides, at the

2-fold R. rates, induced significant increases of the GOT specific activity, where lufenuron and pymetrozine recorded the highest value of general means (19.62 and 16.67 U/L/mg protein/min) respectively.

The means could significantly be arranged as follows: lufenuron (19.62) at 2-fold R. rate ≥ pymetrozine (16.67) at double rate ≥ abamectin (15.03) at double rate ≥ mefenoxam plus at 2-fold R. rate (13.54) ≥ fluazifop-P-butyl at 2-fold R. rate (10.75) ≥ M.A.P. (8.91) ≥ pymetrozine at R. rate (7.02) ≥ mefenoxam plus at R. rate (6.38) = lufenuron at normal rate (5.95) = fluazifop-P-butyl at R. rate (5.82) = abamectin at R. rate (5.79) = Microbine (5.59) ≥ untreated check (4.13 U/L/mg protein/min).

The same trend was obtained with increase% of GOT specific activity at the 2-fold R. rate treatments of lufenuron, pymetrozine, abamectin and mefenoxam plus showing the highest values as follows: (375.12, 303.69, 263.92 and 227.85%), respectively (Fig. 4). Reference to these results at the recommended rate of application, the agrochemicals with a little of exception (M.A.P.), caused transitional effects on GOT specific activity of earthworm, immature stage.

Table (5). Residual effect of certain agrochemicals on GOT specific activity of earthworm, immature stage of *A. caliginosa* in soil

Agro-chemicals	Rate	Specific activity (U/L/mg protein/min) at different week- intervals					
		1st week	2nd week	3rd week	4th week	General mean	% increase of general mean
lufenuron	R	3.60 f± 0.1	7.55 cd±0.2	6.65 gh±1.2	6.01 fg±0.6	5.95 ef	44.07
	2 R	16.54 a±0.8	18.52 a± 1.5	19.41 a±1.3	24.02 b±1.4	19.62 a	375.12
pymetrozine	R	6.99 c ± 0.2	4.31 fg±0.21	8.49 de±0.4	8.30 ef±1.3	7.02 def	70.04
	2 R	10.42b± 0.9	7.64cd± 1.4	15.11 b± 0.4	33.52 a±1.4	16.67 ab	303.69
abamectin	R	4.51 ef± 0.1	2.65 g ± 0.1	7.89def±0.8	8.12 ef ±0.7	5.79 ef	40.25
	2 R	10.24 b± 0.1	19.76 a± 1.1	12.61 c±0.1	17.51 c±1.4	15.03 b	263.92
Fluazifop-p-butyl	R	6.10 cd± 0.1	5.13 ef± 0.5	5.24 h± 0.1	6.80 fg± 0.5	5.82 ef	40.86
	2 R	6.33 cd± 0.1	11.43 b± 1.1	6.66 gh± 0.3	18.56 c± 0.8	10.75 cd	160.17
Mefenoxam- plus	R	5.15 de± 0.1	5.09 ef± 0.4	3.30 h ± 0.1	9.99 e ± 0.3	6.38 ef	54.54
	2 R	11.25 b± 0.1	9.49 bc± 0.9	8.61 de± 1.1	24.81 b± 0.0	13.54 bc	227.85
M.A.P.	R	5.55 e ± 0.1	7.09 de± 0.2	8.99 d± 0.4	13.99 d± 0.8	8.91 de	115.62
Microbine	R	4.16 ef± 0.2	3.48 fg± 0.2	7.4defg±0.3	7.31 fg± 0.4	5.59 ef	35.29
Check	–	3.47 f±0.0	2.49 g ± 0.3	5.06 gh± 0.5	5.50 g ± 0.5	4.13 f	–

* In a column, means followed by the same letter(s) are not significantly different at 0.05 probability level.
* R: Recommended rate * 2 R: 2-fold recommended rate * M.A.P.: Mono ammonium phosphate

GPT activity affected by the tested agrochemicals was recorded in Table (6) and Fig. (4). It is clear that most of the agrochemical treatments caused significantly increase in the GPT specific activity of earthworm compared with untreated check where, after the 1st week of treatment, the highest elevation was occurred by lufenuron followed by pymetrozine at the 2-fold recommended rate (5.20 and 5.12 U/L/mg protein/min), respectively. Also, after the 2nd week of treatment, lufenuron had the highest enhancing on GPT specific activity (8.9) followed by abamectin (7.49), at the double rate. At the 3rd and the 4th week, pymetrozine at 2-fold R. rate caused the highest increase in GPT specific activity (8.12 and 10.19 U/L/mg protein/min), respectively as shown in Table (6).

Table (6). Residual effect of certain agrochemicals on GPT specific activity of earthworm, immature stage of *A. caliginosa* in soil

Agro-chemicals	Rate	Specific activity (U/L/mg protein/min) at different week- intervals					
		1st week	2nd week	3rd week	4th week	General mean	% increase of general mean
lufenuron	R	2.19 ef± 0.1	5.72 de±0.4	3.3def±0.5	2.39gh±0.1	3.41 de	84.30
	2 R	5.20 a±0.3	8.90 a±0.6	5.13 b±0.6	5.23 c± 0.1	6.12 ab	230.99
pymetrozine	R	3.31 c ± 0.1	3.30 ef±0.2	3.63 de±0.1	3.59 f± 0.4	3.46 de	87.14
	2 R	5.12 a ± 0.7	5.84 cd ±1.0	8.12 a± 0.3	10.19 a±0.2	7.32 a	296.08
abamectin	R	2.50 de±0.1	2.56ghi±0.1	2.87efg±0.3	2.72 g± 0.3	2.66 de	44.11
	2 R	4.61 b ± 0.2	7.49 ab±0.4	4.70 bc±0.2	6.51 b± 0.6	5.83 ab	215.43
Fluazifop-p-butyl	R	2.59 de±0.1	3.81 fg± 0.2	2.75efg±0.1	1.96 h± 0.1	2.78 de	50.34
	2 R	2.79 cd±0.1	4.36 ef± 0.3	3.35 cd±0.1	4.46 dc±0.0	3.87 cd	109.20
mefenoxam plus	R	2.82 cd±0.8	3.56fgh±0.0	2.48 fg±0.2	2.83 g±0.1	2.92 de	58.19
	2 R	4.51 b± 0.1	7.21 bc±0.7	4.65 bc±0.6	5.64 c ± 0.1	5.50 bc	197.83
M.A.P.	R	2.78 d±0.1	3.71 fg±0.0	5.15 b±0.4	4.50 d±0.3	4.04 cd	118.40
Microbine	R	1.83 fg±0.1	2.22 hi± 0.1	3.14defg±.0	3.77 ef ±0.2	2.74 de	48.31
Check	–	1.69 g±0.1	1.60 i± 0.1	2.21 g±0.3	1.89 h±0.1	1.85 e	–

* In a column, means followed by the same letter(s) are not significantly different at 0.05 probability level.
* R: Recommended rate * 2 R: 2-fold recommended rate * M.A.P.: Mono ammonium phosphate

Pymetrozine recorded the highest elevation in the means of GPT specific activity using the double rate of application, in comparison with check; (7.32) and (1.85), respectively. However, abamectin, Microbine, fluazifop-P-butyl and mefenoxam plus at R. rate caused a slight enhancing in GPT specific activity, (2.66), (2.74), (2.78) and (2.92), respectively. The general means could significantly be arranged as follows: pymetrozine at the 2-fold R. rate (7.32) > lufenuron at the 2-fold R. rate (6.12) = abamectin at the double rate (5.83) = mefenoxam plus at the double rate (5.50) > M.A.P. (R. rate) (4.04) = fluazifop-P-butyl at 2-flod R. rate (3.87) ≥ pymetrozine at R. rate (3.46)≥ lufenuron at normal rate (3.41) ≥ mefenoxam plus at R. rate (2.92)≥ fluazifop-P-butyl at R. rate (2.78) ≥ Microbine at normal rate (2.74) = abamectin at R. rate (2.66)≥ check (1.85) U/L/mg. protein/min.

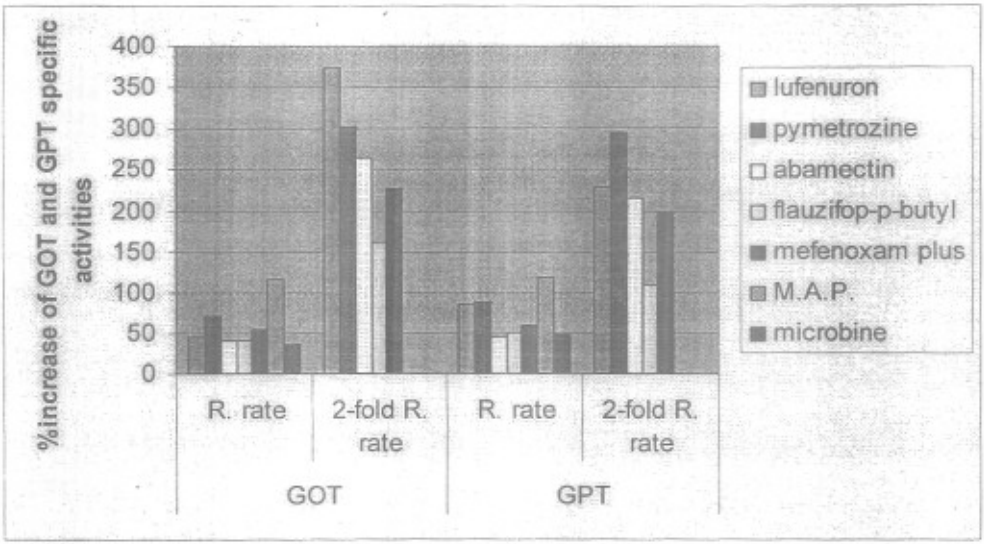

Fig. (16): Residual effect of certain agrochemicals on GOT and GPT specific activity of earthworm, immature stage of *A. caliginosa*

The increase% of GPT specific activity with 2-fold R. rate treatments of pymetrozine, lufenuron, abamectin and mefenoxam plus (Fig. 4) recorded the highest values as follows (296.08, 230.99, 215.43 and 197.83%), respectively.

It could be concluded that the immature stage of *A.caliginosa* is more sensitive to the agrochemicals than the adult stage. Reviewing the obtained results, it could be concluded that, there is a negative relation between the

activity of the two enzymes and the soluble protein content. The present observation are in line with recorded by Abdel-Ghany *et al.* 1985, Ahmed *et al.*(1991), Ismail *et al.* ($1997_{a, b}$) and Beltagy (2000).

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الملخص العربي

الأثر الباقي لبعض الكيميائيات الزراعية على المحتوى الكلي للبروتين الذائب و الناقلين لمجموعة الأمين في أنسجة GPT و GOTالنشاط النوعي لأنزيمي دودة الأرض

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هدفت الدراسة الى تقدير الأثر الباقي لكل من الجرعة الموصى بها و ضعفها لكل من أبامكتين، فلوزيفوب-ب-بيوتايل، ليوفينيورون، ميفينوكسام بلاس و بايميتروزين و كذلك الجرعة الموصى بها من السماد المعدني (مونو أمنيوم فوسفات) و السماد الحيوي (ميكروبين) على كل من الطور الناضج و غير الناضج من دودة الأرض.أدى استخدام ضعف الجرعة لمعظم الكيميائيات المستخدمة إلى إحداث تأثيرات ضارة من خلال خفض محتوى البروتين الذائب، بينما كان للجرعة الموصى بها تأثير طفيف. حيث أظهر ضعف الجرعة من فلوزيفوب-ب-بيوتايل، ليوفينيورون، ميفينوكسام بلاس أعلى نسبة خفض في الطور البالغ (٥٠,٧٧، ٤٨,٢٩، ٤٧,١٢%) على التوالي. أما في الطور غير البالغ تسبب بايميتروزين و أبامكتين و ، ميفينوكسام في إحداث خفض قدره ٥٨,٤٣، ٥٣,٣٠، ٥٣,٠٧% على الترتيب. بينما أدى استخدام السماد المعدني إلى إحداث أعلى نسبة خفض لمحتوى البروتين الذائب لكل من الطور البالغ و غير البالغ (٤٤,٢٠، ٤٠,١٨%) على التوالي مقارنة بكل الكيميائيات المختبرة.أثرت الجرعة الموصى بها للكيميائيات المستخدمة (باستثناء السماد المعدني) تأثيرا طفيفا على GOT و GPT. بينما أدت الجرعة المضاعفة إلى زيادة النشاط النوعي للأنزيمين لكلا مرحلتي النمو. في مرحلة الطور البالغ، كانت أعلى زيادة في نشاط GOT في معاملة فلوزيفوب-ب-بيوتايل، ميفينوكسام بلاس و أبامكتين (٣٥٤,٤٢، ٢٢٣,١٥، ٢١٣,٨٤%)، بينما ليوفينيورون و أبامكتين أظهرا أعلى نشاط في GPT (٢٢٢,٦٨، ٢٠١,٨٠%). أما في حالة الطور غير البالغ أدى استخدام الجرعة المضاعفة من ليوفينيورون،

و أبامكتين و ، ميفينوكسام بلاس الى أعلى زيـــادة فـــي نـــشاط GOT (٣٧٥,١٢، ٣٠٣,٦٩، ٢٦٣,٩٢، ٢٢٧,٨٥%)، في حين أظهر بايميتروزين، ليوفينيورون، أبامكتين و ميفينوكسام بلاس أعلى زيادة في نشاط GPT (٢٩٦,٠٨، ٢٣٠,٩٩، ٢١٥,٤٣، ١٩٧,٨٣%) على الترتيب.كما أشارت الدراسة إلى أن الطور الناضج جنسيا كان أكثر حساسية للكيماويات المستخدمة و ذلك قابلية المبيدات المختبرة علي التحطم في التربة، حيث لم يكن هناك تأثير واضح لها بعد ثلاثة أسابيع حتى مع استخدام ضعف الجرعة.